***BRAF* alterations** (Non-core)

Reason/Evidentiary Support

BRAF Mutation

The *BRAF* V600E mutation in exon 15, which is the most common *BRAF* alteration, affects a large variety of CNS tumours. It has been reported in 96% of papillary craniopharyngiomas[1], 65-75% of pleomorphic xanthoastrocytomas (PXA) with and without anaplasia[2], 25-60% of gangliogliomas, 20–25% of dysembryoplastic neuroepithelial tumours (DNET), and 7% of pilocytic astrocytomas (PA), especially those in supratentorial locations.[1,2,3,4] *BRAF* mutation has been also detected in about one-half of epithelioid glioblastomas and, in up to 25% of diffuse astrocytic gliomas in children and young adults.[5] The detection of a *BRAF* mutation has diagnostic implications in specific tumours such as PXA, ganglioglioma, DNT, or epithelioid glioblastoma. Moreover, the detection of the mutation can help to distinguish a ganglioglioma from the cortical infiltration of a diffuse glioma. Besides its diagnostic value, *BRAF* mutation has therapeutic implications as targeted therapies against mutated BRAF V600 protein have been recently developed, including in settings such as *BRAF*-mutant craniopharyngioma.[6] In paediatric low-grade gliomas, *BRAF* V600E mutation has been linked to poor response to conventional cytotoxic therapy and poor prognosis.[7] In routine settings, BRAF V600E can be identified by IHC (see below) or by molecular approaches such as Sanger sequencing, high-resolution melting analysis, pyrosequencing, allele-specific quantitative polymerase chain reaction (ASQ-PCR), and next-generation sequencing (NGS).[8] Although Sanger sequencing is a well-established tool to detect *BRAF* V600E and other rarer *BRAF* mutations, it has a detection threshold of 20% (of mutated alleles). This high threshold reduces the relevance of this technique in samples that contain a minority of mutated cells. Molecular methods with much lower thresholds, such as ASQ-PCR, digital PCR, or NGS, are more sensitive although precise cut-offs for mutant allele frequency have not been defined.

BRAF V600E Expression (Immunohistochemistry)[9]

Immunohistochemistry is a commonly used method to detect the BRAF V600E protein in FFPE tissue in CNS tumours.[10,11] Two monoclonal antibodies (clone VE1 and clone V600E) against BRAF V600E are commercially available. Clone VE1 is the most widely used and is sensitive and specific.[12] The concordance between immunohistochemistry and detection of *BRAF* V600E mutation by molecular genetic techniques demonstrates variability between studies in different types of neoplasms, but the overall concordance is strong.[12] Immunohistochemistry plays a key role when FFPE material available is not sufficient for molecular genetic analysis and when low tumour cell content may lead to false-negative results. The presence of nonspecific staining is a potential pitfall, which could lead to false-positive results, and light staining can lead to false-negative interpretations.

BRAF Rearrangement/Duplication

Circumscribed duplication of the *BRAF* locus is a common copy number variation that occurs in PAs of the cerebellum, hypothalamus, or optic chiasm, but may occur in PAs from other sites as well. Chromosome 7q34 gain has been characterised as a *BRAF* duplication with a tandem insertion in the *KIAA1549* gene.[13] Fusion genes containing *BRAF* variants activate the MAPK signalling pathway, which appears to be the key signalling pathway in the development of PA. The major alterations leading to constitutive activation of MAPK in PAs are gene fusions and point mutations involving *BRAF*. Fusions between *KIAA1549* and *BRAF* are the most frequent genetic change in PAs (>70 %) and occur in almost all anatomical locations, although most frequently in the cerebellum and less frequently at other sites. The most common fusion is between *KIAA1549*-exon 16 and exon 9 of

BRAF, followed by 15-9, and 16-11. Much rarer fusions involving *BRAF* or *RAF1* have also been found. Identification of the *KIAA1549-BRAF* fusions has been used as a diagnostic marker for PAs. It has been observed in pilomyxoid astrocytoma, ganglioglioma and in the recently described diffuse leptomeningeal glioneuronal tumour (DLGNT).[14 15] *KIAA1549-BRAF* fusions, while all coding for a fusion protein that includes the activating *BRAF* kinase domain, can be derived from at least nine different fusion site combinations. This makes reverse transcriptase polymerase chain reaction (RT-PCR) a difficult method to identify or exclude all variants of the fusion gene. Fluorescence in situ hybridisation (FISH) analysis, which demonstrates the tandem duplication at 7q34, is an indirect way to indicate the presence of a *KIAA1549-BRAF* fusion. However, *BRAF* copy number gains due to trisomy 7 or whole 7q gains are common in diffusely infiltrating astrocytomas including glioblastomas, and should not be mistaken as circumscribed *BRAF* duplication or *BRAF* fusion. A method that may identify all types of *BRAF* and *RAF1* fusion variants in a single experiment is RNA sequencing by NGS.

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